Separatum EXPERIENTIA *20*, 310 (1964)
Birkhäuser Verlag, Basel (Schweiz)

Age Factor in the Maturation of Collagen. Intramolecular Linkages in Mildly Denatured Collagen[1]

The relative amount of soluble collagen decreases in the tissues with advancing age[2], but nothing is known about the eventual qualitative changes in its composition, e.g. in the ratio of cross-linked β-units to single-chain α-units. We have evidence that the intermolecular cross-linking increases with age in skin[3]. This report describes information on the 'subunit'-pattern, and consequently on the intramolecular cross-linking of collagen.

The ages of the rats of Wistar strain were known to the accuracy of a few days. From the tail tendon fibres the samples were prepared by extraction for 30 min at +40°C in pH 4.5, 0.01 *M* acetate buffer (100-fold v/w). The suspension was centrifuged at 16000 rpm and the supernatant was used for the electrophoretic analyses. This material is not identical with denatured soluble collagen, obtained by heating soluble collagens. In the present conditions very little is extracted into solution without heating. The dissolved portion was about 20–30%

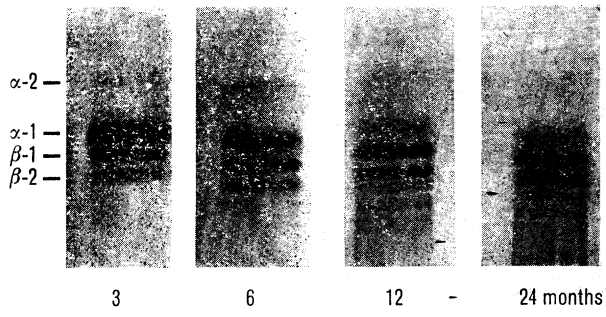

Fig. 1. Starch gel electrophoretic patterns of mildly denatured collagen samples from the tail tendon fibres of rats of indicated ages.

of the whole material, decreasing with age. The ratio of nitrogen to hydroxyproline was rather constant in all the final samples from rats of 6 weeks to 24 months.

The corresponding skin extracts contained much more non-collagenous material. By a chance observation it was found that even the denatured soluble collagen was precipitated by sodium chloride in the final concentration of 15%, w/v[4]. For the purification of the skin material this precipitation was satisfactory.

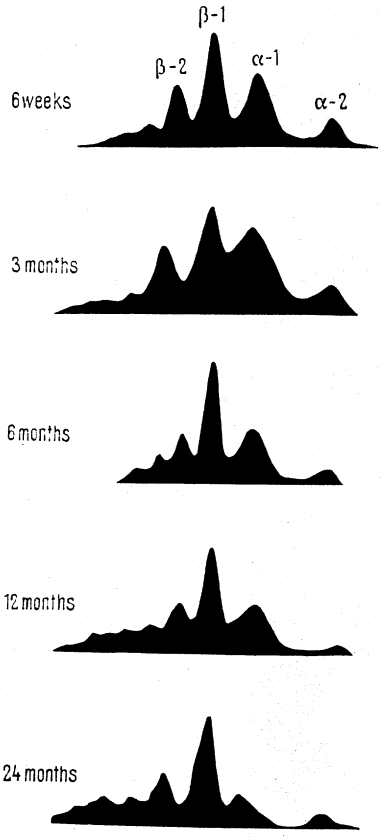

Fig. 2. Densitometric tracings of starch gel electrophoretic patterns of mildly denatured collagen samples from the skins of rats of indicated ages.

The electrophoretic method has been described separately[5]. The results are collected in Figures 1 and 2. It is observed that in the tail tendon fibres the α-units decrease in the relative favour of β-units, especially of β_1. In addition, there appears a large, poorly defined, lagging fraction of unknown composition. In the skin preparations the changes are similar, although less marked. It is known also from the mechanical analysis of cross-links[3], that there is a difference between the rat skin and tail tendon collagen. It remains to be investigated whether the age effects depend on decreased synthesis of new α-units, on a real change in the equilibrium between α- and β-units, or on different rates of formation of the single-chained α-units from larger aggregates on heating.

Preliminary experiments were tried to influence the 'subunit'-pattern *in vitro*. Incubation with homogenized granulation tissue was unsuccessful, as was also treatment with hydroxylamine[6] at pH 7. The treatment with hydrazine was tried at pH 10. It is not surprising that the variation of pH changed the electrophoretic pattern, and hydrazine caused a further blurring of the bands.

Zusammenfassung. In den Extrakten von Schwanzsehnen der Ratten (30 min in Natriumacetatpuffer, pH 4,5, auf +40°C erwärmt) vermehren sich mit zunehmendem Alter die elektrophoretisch getrennten β-Komponenten auf Kosten der α-Komponenten.

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[1] This work has been aided by institutional grants from Sigrid Jusélius Foundation and U.S. Department of Agriculture, Foreign Research and Technical Programs Division.

[2] F. Verzár, Gerontologia *4*, 104 (1960).

[3] E. Kulonen, Leena Mikkonen, and E. Heikkinen, unpublished work and a preliminary abstract in Biochem. J. *89*, 63P (1963).

[4] Similar procedure has been used by V. N. Orekhovich and V. O. Shpiťiter, Biokhimia *23*, 285 (1958).

[5] V. Näntö, J. Maatela, and E. Kulonen, Acta chem. scand. *17*, 1604 (1963).

[6] P. M. Gallop, S. Seifter, and E. Meilman, Nature *183*, 1659 (1959).